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(54) Title: COMPOUNDS OF BIOAVAILABLE IRON WITH ACYLATED OVOTRANSFERRIN OR WITH ACYLATED HYDROLYSIS DERIVATIVES THEREOF

(57) Abstract

Compounds of bioavailable iron with acylated ovotransferrin or with acylated hydrolysis derivatives thereof, useful in the treatment of iron deficiencies.

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COMPOUNDS OF BIOAVAILABLE IRON WITH ACYLATED OVOTRANSFERRIN OR WITH ACYLATED HYDROLYSIS DERIVATIVES THEREOF

The present invention relates to compounds of bioavailable iron with acylated ovotransferrin or with acylated hydrolysis derivatives thereof.

Iron, which is present in all the body tissues, plays a paramount physiological role. The iron requirement is satisfied partially by the use of endogenous iron, deriving from the degradation of old erythrocytes, and partially from the absorption of exogenous iron.

Exogenous iron is absorbed along all the duodenum and the upper part of jejunum and it is accumulated mainly in the liver.

The first pathological symptom of iron deficiency is hypochromic sideropenic anemia, whose primary causes can be of various origin: chronic hemorrhages occurring in case of gastroduodenal ulcers or neoplasias; an insufficient diet or a bad absorption, as in the case with diarrhoea; increased requirements, for example during pregnancy, lactation, infectious diseases and the like; impaired metabolic utilization; particular treatments, such as with ACTH or cortisones.

The administration of iron proved to effectively reduce the iron-related anaemic condition, but it is generally accompanied by undesired side-effects, which are related to the type of vector used for the iron.

The ferro-dextran complex has been suggested for the intramuscular administration, whereas the ferro-

dextrin complex is used for the intravenous administration. The side effects of both said complexes can be allergic reactions, temperature rises, tachycardia, leukocytosis, lymphoadenopathy, in the case of intramuscular treatment, and even anaphylactic shock, thrombophlebitis and circulatory collapse in the case of intravenous treatment.

In the per os treatment, formulations are used based on organic salts (citrate, cholinate, aspartate, gluconate, glycinate, lactate, oxalate, succinate etc.) or inorganic salts (ferric chloride, ferrous sulfate, ferric phosphate etc.) which generally lead to gastrointestinal lesions with necrosis and perforation of the mucous membranes in the most serious cases, and diarrhoea and vomiting. Moreover, the low tolerability makes the administration of suitable amounts of iron difficult. In order to minimize the side effects, the simultaneous intake of food has been suggested, but this is in contradiction with the proven variability of the iron absorption as a function of the composition of food itself and of the degree of the gastric contents.

An alternative to the use of said salts in the oral therapy has been provided by the commercialisation of specialties based on ferritin, which is a ferric globulin representing the most important iron-containing protein in mammals. The commercial product is extracted from horse spleen as a raw material. Ferritin has a 20% iron content in terms of dry weight, it is water soluble and suitable for the oral administration. Ferritin based treatment does not involve the gastrointestinal side effects arising

during the use of the above mentioned iron derivatives, but it has severe restrictions deriving both from the very high cost of the raw material and mainly from the limited availability of extraction sources.

Therefore an attempt was carried out to use other proteins from animals (serum proteins, organ proteins, ovoalbumin, lactoproteins) or from vegetables (soy proteins) as iron carriers. However, the interaction between ferric salts and the above mentioned proteins leads to the formation of ferro-protein derivatives whose therapeutic interest is undermined by a series of negative characteristics, including:

- the insolubility of the derivatives obtained when the percentage of iron linked to the protein reaches values greater than 0.5%;
- the difficulty or even the impossibility of evaluating what fraction of the total iron content, under such conditions of insolubility, is actually linked to the protein and what fraction is co-precipitated in the form of hydrated oxides which may cause severe gastric lesions;
- the lack of homogeneity and compositional stability of these derivatives with respect to iron.

Subsequently, it has been found (see Italian Patent n. 1150213 in the Applicant's name) that, by carrying out a succinylation of the above mentioned proteins and reacting them with iron, ferroprotein derivatives could be obtained which have a fairly good iron content, are stable and sufficiently soluble at pH values above 5, and are able to supply therapeutically

acceptable iron concentrations when administered orally. However, since said proteins have a varying composition, it is very difficult to obtain compounds having a constant iron content. Moreover, even though compounds can theoretically be obtained with a fairly good iron content (up to 20%), such an iron content involves an increase in the viscosity of the solution of said products, therefore up to now such products are commercialized having an iron content of only 5% by weight.

WO 91/07426 discloses a very soluble iron-acylated albumin compound, but, even though the use of different types of albumin is stated to be effective, the best results are achieved with bovine serum-albumin, which yields a compound with a 10% iron content, above which value the solubility of the compound decreases, thus lowering the therapeutic value. Recently, the use of all of the products of bovine origin has severely been restricted by the dramatic problem of the virus of bovine spongiform enkephalitis (BSE) which has already caused some therapeutically interesting substances, such as ferritin, to be withdrawn from the market.

As a consequence, the interest of researchers has been focalized on proteins of a different origin.

Now it has surprisingly been found that, among all of the proteins useful as iron carriers in the martial therapy, ovotransferrin (also named conalbumin), suitably acylated, gives compounds having a higher iron content than other ferro-protein compounds, while keeping those viscosity and solubility parameters which make therapeutically acceptable the compound, therefore

ensuring a larger iron supply to the patient without any of the undesired side effects typical of said therapy occurring.

The present invention relates to compounds of bioavailable iron with acylated ovotransferrin or with acylated hydrolysis derivatives thereof.

Preferably, the acyl moiety of the compound consists of a dicarboxylic acid derivative such as malonic, succinic, methylmalonic, ethylmalonic, acetylmalic, acetylglutamic, acetylaspartic, glutaric acids and the like. Preferred carboxylic acid derivatives are the succinic and acetylaspartic acid derivatives.

The compounds of the present invention have an iron content from 3 to 20% by weight. Preferably the iron content of said compounds is 11% by weight at least.

Said compounds are suitable as active ingredients for the preparation of pharmaceutical compositions which can be used in the oral treatment of anemias and in all the pathological conditions caused by an iron lack in mammals and in man. Therefore another object of the invention is provided by the use of the present compounds for the preparation of medicaments useful in said pathologies. Pharmaceutical forms suitable for the oral administration of the compounds of the present invention are, for example, tablets, sugar-coated tablets, capsules, powders, granulates, syrups, suspensions and solutions.

The present invention is illustrated in further detail by the following non-limiting example.

EXAMPLE

5 g of ovotransferrin are dissolved in 100 ml of water containing 3 g of $KHCO_3$, the clear solution is added with 2.5 g of succinic anhydride, in subsequent portions and adjusting pH to values ranging from 5 to 8 by addition of NaOH. The mixture is left to react for 2 hours at room temperature, then, after acidification to pH 3.4, a precipitate is obtained which is recovered by centrifugation, purified adjusting pH to 7.5 by addition of NaOH and subsequently reprecipitated at pH 3.4. By centrifugation a solid is recovered which is dried under vacuum. The dry solid is resuspended in distilled water and dissolved by addition of NaOH to pH 8, to obtain a final solution of 0.04 g of protein/ml.

Said solution, having a very high viscosity, is added with a solution of ferric chloride so as to obtain a weight ratio of succinylated protein to Fe^{3+} of 10:1. Under said conditions, pH decreases to 2.6 and a precipitate forms which is recovered by filtration, then redissolved in water and added with NaOH until complete dissolution (pH 7.5). After dialysis against water to remove sodium chloride, the solid product is recovered by lyophilization.

The compound yield is 33% by weight of the starting protein and the iron content is 11%.

The product of the above example was administered orally to groups of rats with strong sideropenic anemia, experimentally induced by feeding the animals with an iron-free diet from the pre-natal time to the one of the test. The administered compound dose was 1 mg/kg iron.

A group was treated with placebo. One or two hours after the treatment, the animals were killed with ether, the blood was collected, the serum was prepared and sideremia was evaluated by means of a commercial kit. The table below summarizes the means ± S.E. of the values obtained in 6 animals.

Treatment	Withdrawal time	Serum Fe ug/100 ml
Placebo	1 hour	60.9± 3.3
Fe ovotransferrin	1 hour	358.2±30.9
Fe ovotransferrin	2 hours	484.9±46.4

CLAIMS

1. A compound of bioavailable iron with acylated ovotransferrin or with an acylated hydrolysis derivative thereof.

2. A compound according to claim 1, wherein the acyl moiety is a dicarboxylic acid derivative.

3. A compound according to claim 2, wherein the dicarboxylic acid derivative is a succinic or an acetylaspartic acid derivative.

4. A compound according to claim 1, wherein the iron content is from 3 to 20% by weight.

5. A compound according to claim 1 or 4, wherein the iron content is at least 11% by weight.

6. The use of the compound of claim 1 for the preparation of medicaments useful in the iron deficiencies.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/02489

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/14

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	BIOCHEMISTRY vol. 4, no. 6, June 1965, EASTON, PA US pages 998 - 1005 H. BUTTKUS, 'Chemical modifications of amino groups of transferrins: ovotransferrin, human serum transferrin and human lactotransferrin.'	1-5
Y	see the whole document ---	6
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Y	see the whole document ---	6

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° Special categories of cited documents : [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 FEBRUARY 1993	10. 03. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT FFICE	ORVIZ DIAZ P.

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

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Y	see the whole document	6
Y	US,A,4 493 829 (G. SPORTOLETTI) 15 January 1985 see the whole document, especially see claim 12; example 4 & IT,A,1 150 213 (cited in the description)	1-6
Y	WO,A,9 107 426 (ITALFARMACO S.P.A) 30 May 1991 see claims (cited in the description)	1-6
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9202489
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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/02/93
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